VERIFICATION OF TRANSLATION

I, Helmut Madl,
translator to

Winter, Brandl, Fürniss, Hübner,
Röss, Kaiser, Polte ▪ Partnerschaft
Patent Attorneys and Attorneys at Law
of 85354 Freising, Germany,

do solemnly and sincerely declare that I am conversant with the English and German languages and am a competent translator thereof, and that to the best of my knowledge and belief the attached is a true and correct translation of a German Patent and Trademark Office Priority Certification
in respect of **German Patent Application No. 10 2005 001 889.0**.

September 10, 2010

H. Madl
....
(Helmut Madl)

FEDERAL REPUBLIC OF GERMANY

Priority certificate on filing a patent application

Serial number: 10 2005 001 889.0

Application date: January 14, 2005

Applicant/Proprietor: SIRS-Lab GmbH, 07745 Jena/DE

Title: *Verfahren zur Steigerung der Bindungskapazität -und effizienz eines Proteins, das nicht methylierte CPG-Motive enthaltende DNA spezifisch bindet* [Method of enhancing the binding capacity and efficiency of a protein specifically binding DNA containing non-methylated CPG motifs]

IPC: C 12 Q 1/68

The annexed sheets are a true and exact reproduction of the original papers of this patent application.

Munich, March 2, 2005
By authority of
The President of the
German Patent and Trademark Office
(signed)
Dzierzon

SIRS-Lab GmbH
Attorney's file: PAT 3696/031

January 14, 2005
H/18/kt

Description

Method of enhancing the binding capacity and efficiency of a protein specifically binding DNA containing non-methylated CPG motifs

The invention relates to a method of enhancing the binding capacity and efficiency of a protein which binds non-methylated cytidine-phosphate-guanosine dinucleotides (CpG motifs) of a DNA, the use of a method for separating and/or enriching DNA containing non-methylated CpG motifs, as well as to a kit for carrying out said method.

Infections caused by bacteria are one of the most frequent causes of inflammatory diseases. Early detection of the bacterial pathogens is crucial for the prognosis of the course of the disease as well as, in particular, for timely selection of suitable therapeutic measures.

For the detection of bacterial pathogens, use is primarily made even today of different culture-dependent methods. As a result of the disadvantages of these methods, increased efforts were undertaken to find alternatives, especially during the past decade, simultaneously with the rapid technological development in molecular biology. First reports on the use of culture-independent methods of detecting bacterial pathogens, based on the principle of the polymerase chain reaction (PCR), date back to the early 1990s. Thus, for instance, Miller and colleagues (Miller N J Clin Microbiol. 1994 (Feb;32(2):393-7) were able to show that culture-independent methods are superior to the classical techniques of cultivation and microscopy in the detection of *mycobacterium tuberculosis*. Recently, however, further molecular-biological methods based on the detection of pathogen-specific nucleic acids have gained importance (e.g., M. Grijalva et al. Heart 89 (2003) 263-268; Uyttendaele M et al. Lett Appl Microbiol. 2003;37(5):386-91; Saukkoriipi A et al. Mol Diagn. 2003 Mar;7(1):9-15; Tzanakaki G et al. FEMS Immunol Med Microbiol. 2003 Oct. 24;39(1):31-6).

Besides the high specificity of such molecular-biological methods, the reduced time expenditure is to be mentioned as a substantial advantage over conventional culture-dependent methods. Nevertheless, the sensitivity of direct detection of prokaryotic DNA from body fluids and not from pre-treated testing material as compared to culturing of

microorganisms has been much too low so far. At best, an amount of nucleic acids of bacteria sufficient for the direct detection of pathogens from testing material which is not pre-treated is achieved in the area of 16S-rRNA analysis by means of PCR of the 16S region on the bacterial chromosome and the subsequent sequence analysis of the PCR fragment, because in most cases several copies for the segment coding the 16S-rRNA are situated on the chromosome. The direct specific detection of pathogens by means of 16S-rRNA analysis requires that only one pathogen species is present in the sample to be examined. If there are different pathogen species in the sample, specific detection by way of sequencing of the 16S-rRNA region is not possible because the primers used are universal for most bacteria. Furthermore, the pathogens to be detected must be in the metabolic phase and express enough 16S-rRNA. As a rule this can not be assumed to be the case, in particular in patients subject to a calculated antibiotic therapy.

Moreover, an expression of certain pathogenicity factors of bacteria does not occur at all times despite the fact that the corresponding genes are present in the bacterial genome. As a result, false negative results are transmitted to the clinical physician. Selective antibiotic therapy may thus be initiated either not at all or much too late. In such cases, the physician has to rely on his knowledge gained by experience and on general guidelines (such as those of the Paul Ehrlich Foundation) and will therefore effect a much too general antibiotic treatment. The unspecific use of antibiotics bears a number of risks, not only for the individual patient (such as unnecessary side effects in the form of renal damage etc.), but also for the entire society (e.g., the development of additional antibiotic resistances such as MRSA (methicilline-resistant *Staphylococcus aureus*, etc.). Therefore, the detection of clinically meaningful pathogenicity factors and resistances of bacteria on the chromosomal level and on the plasmid level, i.e. ultimately on the DNA level, provides considerable advantages for the diagnosis of many infectious diseases but also of sepsis. This applies even more because a distinction between pathogenic and commensal bacteria can also be made on this level.

Most frequently, the detection of pathogen-specific nucleic acids is effected by nucleic acid amplification techniques (NAT), such as the amplification of the prokaryotic DNA by means of the polymerase chain reaction (PCR) or the ligase chain reaction (LCR), respectively. The high specificity and fast availability of the results is contrasted by the susceptibility to interference by contamination or by strongly reaction-inhibiting factors in clinical samples.

In a conventional PCR detection method, successful detection of pathogens in the blood requires at least 1 target DNA of the pathogen to be present in 10 µl of blood. This

corresponds to approximately 100 targets in 1 ml of blood or 1,000 targets in 10 ml of blood, respectively.

Things are different with the blood culture for the detection of pathogens causing an infection. In this case, the lower detection limit is approximately 3-5 bacteria per 10 ml of blood.

This detection limit is presently not reached yet by PCR methods, not even by those which have their target sequence in the area of the 16S-rRNA region on the chromosome. Although several regions coding 16S-rRNA - in most cases 3 to 6 - are located on the bacterial chromosome, there is still the prerequisite of at least one molecule of the template DNA being present in the PCR reaction mixture.

Improved diagnostic safety is to be expected of PCR methods whose specific target sequences code for species-specific proteins, either in the chromosome or on plasmids of the microorganisms. The above remarks with respect to the detection limit also apply here. Especially under the influence of an ongoing antibiotic therapy, growth of the pathogens can be decelerated or limited considerably even if the antibiotic employed ultimately does not take effect. This situation is often found especially in patients who are already under antibiotic treatment and in whom disease-causing bacteria can therefore not be grown from the blood cultures or from other samples (such as, for example, tracheal smears, broncho-alveolar lavages (BAL), etc.).

Due to insufficient sensitivity, the detection of pathogen-specific nucleic acids without an amplification step by direct detection of prokaryotic DNA (probe technique, FISH technique) is of diagnostic importance only at a sufficiently high germ count in the test material.

Apart from PCR-inhibiting ingredients in the test material, the essential set of problems in the detection of prokaryotic DNA for the identification of bacterial pathogens in body fluids consists mainly in the low concentration of prokaryotic DNA and the accompanying excess amounts of eukaryotic DNA in contrast with prokaryotic DNA. In this regard, competitive processes in DNA analysis as well as the low quantity of prokaryotic DNA can in particular be regarded as a hindrance to qualitative and quantitative detection of pathogens.

The usual methods of DNA isolation enrich the total DNA of a body fluid such that the ratio of host DNA to microbial DNA may be between $1:10^{-6}$ and $1:10^{-8}$. This difference makes the difficulty in detecting microbial DNA in body fluids quite easy to understand.

Prokaryotic DNA differs from eukaryotic DNA, for example, by the presence of non-methylated CpG motifs (Hartmann G et al., Deutsches Ärzteblatt, Jg. 98/15:A981-A985 (2001). In prokaryotic DNA, CpG motifs are present at a 16-fold excess in comparison with eukaryotic DNA which contains such motifs only temporarily, for example in cancer cells or promoter regions. These motifs are not methylated in prokaryotic DNA, whereas the majority of them are methylated in eukaryotic DNA, which further augments their distinctiveness. Non-methylated CpG motifs are non-methylated desoxycytidylate-desoxyguanylate-dinucleotides within the prokaryotic genome or within fragments thereof.

It is further known that diagnostic statements for cancers can be derived from different methylation patterns within the human DNA (Epigenetics in Cancer Prevention: Early Detection and Risk Assessment (Annals of the New York Academy of Sciences, Vol 983) Editor: Mukesh Verma ISBN 1-57331-431-5). Methylated and non-methylated cytosines in the genome allow tissue-specific but also disease-specific patterns to be identified. The specific methylation patterns of a disease allow, on the one hand, diagnosis at a very early point in time and, on the other hand, molecular classification of a disease and the likely response of a patient to a certain treatment. For detailed information on this, see, for example, Beck S, Olek A, Walter J.: From genomics to epigenomics: a loftier view of life.", Nature Biotechnology December 1999;17(12):1144, on the homepage of Epigenomics AG (http://www.epigenomics.de), or WO 200467775.

Cross at el. showed that it is possible to separate differently methylated genomic human DNA by binding the methylated CpG motifs to a protein (Cross S H, Chariton J A, Nan X, Bird A P, Purification of CpG islands using a methylated DNA binding column, Nat Genet. March 1994;6(3):236-44). Thus, this method serves to bind DNA containing methylated CpG motifs. Sufficient isolation of non-methylated and methylated DNA is not possible for technical reasons, because the protein used also weakly binds non-methylated DNA. It is also not possible with these methods to enrich non-methylated DNA, because the capacity of the protein used is not sufficient to separate non-methylated DNA to a sufficient extent in the case of a high excess of methylated DNA. Further, due to the binding of the methylated DNA, the initial volume in which the non-methylated DNA is present, remains unchanged so that no enrichment is achieved.

Thus it would be desirable to be able to separate non-methylated DNA from methylated DNA and to enrich non-methylated DNA so as to separate prokaryotic DNA from eukaryotic DNA or differently methylated human DNA, respectively, from each other. In addition, it would be desirable and of great interest in terms of health economics if the

isolation and enrichment of non-methylated DNA could also be obtained from a mixture (for example, full blood) which is characterized by a great excess of methylated DNA.

It is known from Voo et al. that the human CpG-binding protein (hCGBP) is capable of binding non-methylated CpG motifs. This publication describes the transcription-activating factor hCGBP which has been shown to play a role in the regulation of gene expression in CpG motifs.

EP 02020904 shows a method which enables isolation and enrichment of prokaryotic DNA from a mixture of prokaryotic and eukaryotic DNA by binding the prokaryotic DNA to a protein which specifically binds non-methylated DNA. From non-published German patent application (Serial no. of the GPTO: 10 2004 010 928.1-41) and from Sachse S. et al. (Using a DNA-binding protein to enrich prokaryotic DNA from a mixture of both, eukaryotic and prokaryotic DNA, Poster Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie, Göttingen 2004) it is known that binding of non-methylated CpG motifs may be achieved through the recombinantly produced protein CpGbP-181 having SEQ ID. No. 1.

It was shown that the CpGbp-181 protein was coupled directly to a matrix. In Sachse et al. the CpGbP-181 protein was coupled directly to bromocyane activated sepharose.

Direct binding of the CpGbP-181 protein does, however, result in undirected binding of this protein across the entire surface of the matrix. This results in restricted mobility of the protein on the matrix surface. Direct binding of the proteins to the matrix can furthermore prevent optimum folding of this protein and reduce the number of free binding sites.

The drawbacks accompanying direct coupling of the CpGbP-181 protein to a matrix lastly result in a reduced binding capacity and efficiency for the non-methylated CpG motifs of a DNA.

The present invention accordingly is based on the object of providing a method [of enhancing] the binding capacity and efficiency of a protein which specifically binds DNA containing non-methylated CpG motifs while having a 25% to 35% homology with the wild type CGPB protein and is shortened with respect to the latter, wherein the binding site for non-methylated CpG motifs is preserved, and thus allows improved separation and/or enrichment of non-methylated DNA from a mixture of methylated DNA and non-methylated DNA.

In the following, the invention shall be described by the example of the recombinantly produced CpGbP-181 protein according to SEQ ID No. 1. The description of the invention relating to the protein CpGbP-181 having SEQ ID No. 1 does not constitute a limitation but only an exemplary application.

According to the invention, this is achieved by indirect coupling of the protein to the matrix. The invention is based on the surprising finding that the binding capacity and efficiency is enhanced by indirect binding of the CpGbP-181 protein.

In the following, the invention will be described by making reference to Fig. 1 and Fig. 2, wherein:

Fig. 1 represents results of the PCR following the enrichment of prokaryotic DNA from a DNA mixture of *Staphylococcus aureus* and human DNA by using coupled CpGbP-181 protein on CNBr sepharose, and

Fig. 2 represents results of the PCR following the enrichment of prokaryotic DNA from a DNA mixture of *Staphylococcus aureus* and human DNA by using coupled CpGbP-181 protein on AH sepharose.

In order to enhance the binding capacity and binding efficiency of the CpGbP-181 protein for non-methylated CpG motifs, the subject matter of the present invention is a method for indirect binding of the protein to the matrix via a spacer. By coupling the protein to the matrix via a spacer, the degree of mobility and the number of free binding sites of the CpGbP-181 protein are increased. Hereby an enhancement of the binding capacity and efficiency is achieved. In addition the quantity of protein used may be reduced.

Spacers within the meaning of the present invention are understood to be molecules which allow a spatial difference between the matrix and the CpGbP-181 protein. Used spacers are molecules such as diamine hexane ($NH_2(CH2)_6$-NH_2) or other inorganic or organic compounds that are known to the person having skill in the art. Antibodies are not to be considered as spacers within the meaning of the invention.

What is referred to as a matrix within the meaning of the present invention are substances which function as carriers for the spacer and the protein. Carrier materials may be, for example, sepharose, pearl cellulose, silica, or similar substances known in the art.

In accordance with a further embodiment, the matrix may be coupled to a solid carrier. This embodiment represents a particularly simple way of enriching DNA containing non-methylated CpG motifs, for separation from the solution may be effected in a particularly simple manner, e.g., by physical removal (e.g. by centrifugation) of the unbound constituents of the mixture from the solution. Suitable carriers include in particular membranes, microparticles and resins or similar materials for affinity matrices. Suitable materials for binding the protein of the invention, as well as - depending on the type of material - for carrying out such binding are well-known to the person skilled in the art.

In accordance with a further embodiment, sepharose is used as a matrix.

In another embodiment, diamine hexane residue (-NH-$(CH_2)_6$-NH-; also referred to as AH) is used as a spacer.

Owing to the enhanced binding capacity and efficiency, a further subject matter of the present invention is a method of separating and/or enriching DNA containing non-methylated CpG motifs, comprising the steps of:

a) coupling the CpGbP-181 protein to a matrix via a spacer,
b) contacting at least one DNA containing non-methylated CpG motifs, which is present in solution, with the CpGbP-181 protein, thus forming a protein-DNA complex, and
c) separation of said complex.

The designation "DNA containing non-methylated CpG motifs" refers to both eukaryotic and prokaryotic DNA. The latter may be purified and dissolved again (e.g., non-methylated DNA isolated from tissues) or may be present directly in the source of origin (e.g. body fluid such as blood, serum, tracheal aspirate, urine, bronchoalveolar lavage, nose smear, skin smear, puncture fluid).

In accordance with a preferred embodiment, the DNA containing non-methylated CpG motifs is prokaryotic DNA, in particular bacterial DNA.

In accordance with another preferred embodiment, the DNA containing non-methylated CpG motifs is humane, genomic DNA.

Separation may be effected by various methods of separating, isolating or enriching DNA-protein complexes or DNA-polypeptide complexes that are well-known to the person skilled in the art. In doing so, use will preferably be made of methods in which the DNA-

binding protein is or is being immobilized to a carrier in order to separate and/or enrich the DNA from the sample solution.

According to a preferred embodiment, separation is followed by a step of separating the DNA from the CpGbP-181 protein from the complex. This may be effected, for example, by conventional methods of DNA purification known to the person skilled in the art. In the most simple case, separation is based on changing the pH value or the salt concentration (e.g., to 1 M NaCl) of the medium/buffer or by adding chaotropic reagents, etc.; i.e., suitable parameters which lead to the dissolution of the protein-DNA complex. Such methods are known to the person skilled in the art.

For the solution for DNA containing non-methylated CpG motifs, any suitable solvent is basically conceivable. However, the method is particularly expedient for enriching DNA containing non-methylated CpG motifs from solutions containing different biomolecular species, in particular different kinds of DNA..

The invention preferably relates to a method of separating and enriching prokaryotic DNA from a mixture of prokaryotic and eukaryotic DNA. In doing so, for example the prokaryotic DNA present in body fluids is separated from the eukaryotic DNA and enriched by specific binding to the protein of the invention. The prokaryotic DNA thus enriched facilitates the detection of prokaryotic pathogens with the aid of molecular-biological methods and may contribute to the diagnosis of diseases caused by pathogenic germs.

The invention further relates to a method of separating and enriching non-methylated genomic DNA from a mixture of non-methylated genomic and methylated genomic DNA. The methylated genomic DNA is separated by binding the non-methylated genomic DNA to the CpGbP-181 protein that is coupled to a matrix via a spacer. This manner of proceeding contributes substantially to a simplified examination of the methylation patters of methylated genomic DNA and allows the diagnosis of diseases exhibiting a specific methylation pattern.

Body fluids within the meaning of the invention are understood to be any fluids originating from the body of a mammal, including humans, in particular such fluids in which disease pathogens may occur, such as blood, urine, liquor, pleural liquid, pericardial liquid, peritoneal liquid, as well as synovial liquid. The description of the invention relating to human blood does not constitute a limitation but only an exemplary application.

Bacterial pathogens are preferably understood to be pathogens of a sepsis, but also any other bacterial pathogens of infections. They may differ from commensal pathogens which are counted among the normal population of the organism and are occasionally also found in test samples from patients but do not have any clinical significance.

Another application of the method of the invention consists in the separation and enrichment of DNA containing non-methylated CpG motifs from methylated DNA by binding of the DNA containing non-methylated CpG motifs to the CpGbP-181 protein which was immobilized on microparticles. In this connection, all microparticles which allow an immobilization of the DNA-binding protein of the invention are suitable. Such microparticles may consist of latex, plastics (e.g. styrofoam, polymer), metal, or ferromagnetic substances. Furthermore, use may also be made of fluorescent microparticles such as those available, e.g., from the company Luminex. After the DNA containing non-methylated CpG motifs has been bound to the proteins according to the invention which are immobilized on microparticles, said microparticles are separated from the mixture of substances by suitable methods such as filtration, centrifugation, precipitation, sorting by measuring the intensity of fluorescence, or by magnetic methods. After separation from the microparticles, the DNA containing non-methylated CpG motifs is available for further processing.

Another application of the method according to the invention consists in the separation and enrichment of DNA containing non-methylated CpG motifs by binding the DNA containing non-methylated CpG motifs to the CpGbP-181 protein which is subsequently separated from other ingredients of the mixture by electrophoresis..

A further application of the method according to the invention consists in the separation and enrichment of DNA containing non-methylated CpG motifs from methylated DNA by binding the DNA containing non-methylated CpG motifs to the CpGbP-181 protein, wherein the CpGbP-181 protein is subsequently bound to corresponding antibodies. The antibodies may be bound to solid or flexible substrates such as glass, plastics, silicon, microparticles, membranes, or may be present in solution. After binding of the DNA containing non-methylated CpG motifs to the CpGbP-181 protein and binding of the latter to the specific antibody, separation from the mixture of substances is effected by methods that are known to the person skilled in the art.

Another application of the method of the invention consists in the detection of DNA containing non-methylated CpG motifs. In this case, enrichment of the DNA containing non-methylated CpG motifs is followed by a step of amplifying this DNA, for which any common amplification methods are suitable (PCR, LCR, LM-PCR etc.).

The invention moreover relates to a kit for separating and/or enriching DNA containing non-methylated CpG motifs by means of one of the above-described methods, said kit containing at least the CpGbP-181 protein, optionally together with further reagents suitable for carrying out said method.

In addition to the CpGbP-181 protein, said kit may contain at least one set of primers which are suitable for amplifying genomic DNA of certain prokaryonts under standard conditions.

The method according to the invention, in particular with the above-described embodiments, has the advantage that owing to the use of a spacer for coupling of the CpGbP-181 protein DNA to a matrix, the binding capacity is enhanced substantially. This allows to concentrate DNA containing non-methylated CpG motifs more highly, whereby in turn the detection sensitivity for the detection of the non-methylated DNA is highly enhanced.

Example

The example shows the enhanced binding properties binding properties of the CpG-bP-181 protein which result from indirect binding of this protein to a matrix via a spacer.

In order to examine the binding properties, prokaryotic DNA was enriched from DNA mixture - mixture of *Staphylococcus aureus* and human DNA - by using the directly coupled CpGbP-181 protein on CNBr sepharose and by using the CpGbP-181 protein on sepharose (hereinafter: AH sepharose) indirectly coupled via a diaminohexyl spacer (AH).

At first, after addition of glutaraldehyde the AH sepharose was incubated 15 minutes at room temperature. Then the AH sepharose was washed with 0.1 mol Na_2HPO_4.

Now, 0.24 mg of the CpGbP-181 protein was added to the matrix. Binding of the CpGbP-181 protein to the AH sepharose was achieved by incubation for 2 hours at room temperature. The excess CpGbP-181 protein was removed.

Following subsequent washing of the CpGbP-181-AH sepharose with 0.1 mol Na_2HPO_4 and addition of 0.1 mol glycine, the CpGbP-181-AH sepharose was incubated during 2 hours at room temperature for saturating free binding sites. After this, the CpGbP-181-AH sepharose was again washed with 0.1 mol Na_2HPO_4.

In order to reduce the Schiff's base and stabilize binding, sodium borohydride was added to the CpGbP-181-AH and incubation performed during 1 hour at room temperature. Then the CpGbP-181-AH sepharose was washed with 0.1 mol Na_2HPO_4.

Storability of the CpGbP-181-AH sepharose at 4°C is achieved by an addition of 20% ethanol. After this the CpGbP-181-AH sepharose was apportioned in columns. The columns prepared with the CpGbP-181-AH sepharose were subsequently washed with TRIS buffer and were available for the separation/enrichment of DNA containing non-methylated CpG motifs.

2) Enrichment of the DNA mixture, subsequent elution of prokaryotic DNA, and determination of the concentration of the prokaryotic DNA by PCR

In every case, the DNA mixture consisted of 330 ng of human DNA and 150 ng of prokaryotic DNA (*Staphylococcus aureus* DNA), respectively.

The DNA mixtures were placed in the columns prepared with CNBr sepharose and with AH sepharose, respectively, and incubated during 1 minute at room temperature. Then the columns were centrifuged and washed with 100 µl of TRIS buffer (10 µM, pH 7). The washing and centrifuging step was repeated 5 times.

The supernatant was removed carefully, and then 100 µl of elution buffer (10 µM TRIS buffer, 0.5 M NaCl, pH 7) each was placed in the columns and centrifuged. The elution step was repeated 5 times.

Now the single fractions of each sample were precipitated by addition of 10 µl of 3 M sodium acetate and 250 µl of ethanol, followed by mixing and centrifuging (15 min at 15000 g). The supernatant was poured away carefully, and the pellet was washed with 1 µl of ethanol (70%) and centrifuged during 5 minutes at 15000 g. After this the supernatant was removed again, and the pellet was dried in a vacuum centrifuge and received in 30 µl of DEPC water.

Of this, 5 µl each was utilized for the detection by PCR. For the PCR, universal primers were used for the 16s RNA gene. After performing the PCR, 15 µl each of the single fractions were placed on a 2% agarose gel.

Fig. 1 (direct binding of the CpG-181 protein to CNBr sepharose) and Fig. 2 (indirect binding of the CpG-181 protein via a spacer (AH) on sepharose) shows the PCR results for the individual fractions. It may be seen clearly that more prokaryotic DNA could be

enriched by using the AH spacer (Fraction 1, Elution fraction). This characteristic improvement of the binding properties may be made use of for the methods of the invention.

SEQUENCE LISTING

<110> SIRS-Lab GmbH

<120> Method of enhancing the binding capacity and efficiency of a protein specifically binding DNA containing non-methylated CPG motifs

<130> Pat 3696/31

<140>
<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 181
<212> PRT
<213> Homo sapiens

<400> 2
```
Gly Gly Gly Arg Lys Arg Pro Val Pro Asp Pro Asn Leu Gln Arg Arg
  1               5                  10                  15

Ala Gly Ser Gly Thr Gly Val Gly Ala Met Leu Ala Arg Gly Ser Ala
             20                  25                  30

Ser Pro His Lys Ser Ser Pro Gln Pro Leu Val Ala Thr Pro Ser Gln
         35                  40                  45

His His Gln Gln Gln Gln Gln Gln Ile Lys Arg Ser Ala Arg Met Cys
     50                  55                  60

Gly Glu Cys Glu Ala Cys Arg Arg Thr Glu Asp Cys Gly His Cys Asp
 65                  70                  75                  80

Phe Cys Arg Asp Met Lys Lys Phe Gly Gly Pro Asn Lys Ile Arg Gln
                 85                  90                  95

Lys Cys Arg Leu Arg Gln Cys Gln Leu Arg Ala Arg Glu Ser Tyr Lys
            100                 105                 110

Tyr Phe Pro Ser Ser Leu Ser Pro Val Thr Pro Ser Glu Ser Leu Pro
        115                 120                 125

Arg Pro Arg Arg Pro Leu Pro Thr Gln Gln Gln Pro Gln Pro Ser Gln
    130                 135                 140

Lys Leu Gly Arg Ile Arg Glu Asp Glu Gly Ala Val Ala Ser Ser Thr
145                 150                 155                 160

Val Lys Glu Pro Pro Glu Ala Thr Ala Thr Pro Glu Pro Leu Ser Asp
                165                 170                 175

Glu Asp Leu Pro Leu
            180
```

SIRS-Lab GmbH
Attorney's file: PAT 3696/031

January 14, 2005
H/18/kt

Claims

1. A method of enhancing the binding capacity and efficiency of a protein which specifically binds DNA containing non-methylated CpG motifs while having a 25% to 35% homology with the wild type CGPB protein and is shortened with respect to the latter, wherein the binding site for non-methylated CpG motifs is preserved, with binding of this protein to a matrix taking place via a spacer.

2. The method according to claim 1, wherein the protein includes the amino acid sequence according to SEQ ID No. 1.

3. The method according to any one of claims 1 or 2, wherein a diaminohexane residue is used as a spacer.

4. The method according to any one of claims 1 to 3, wherein sepharose is used as a matrix.

5. A method of separating and/or enriching DNA containing non-methylated CPG motifs with a protein according to any one of claims 1 or 2, including the steps of:

   a) coupling the proteins to a matrix via a spacer,
   b) contacting at least one DNA containing non-methylated CpG motifs, which is present in solution, with the protein, thus forming a protein-DNA complex, and
   c) separation of said complex.

6. The method according to claim 5, wherein separation is followed by a step of separating the DNA containing non-methylated CPG motifs from the protein from the complex.

7. The method according to any one of the preceding claims, wherein the DNA containing non-methylated CPG motifs is prokaryotic DNA.

8. The method according to claim 7, wherein the prokaryotic DNA is bacterial DNA.

9. The method according to any one of the preceding claims 1-6, wherein the DNA containing non-methylated CPG motifs is human genomic DNA.

10. The method according to any one of the preceding claims, wherein a solid carrier is used as a matrix.

11. The method according to claim 10, wherein the carrier is provided as a matrix, microparticles, or a membrane.

12. The method according to any one of claims 5 to 11, wherein separation is performed with the aid of an antibody or antiserum directed against the protein.

13. The method according to claim 12, wherein separation is performed with the aid of electrophoresis.

14. The method according to any one of claims 5 to 13, wherein the solution contains a mixture of eukaryotic and prokaryotic DNA.

15. The method according to claim 14, wherein the solution is a body fluid or derived therefrom, in particular whole blood, serum, plasma, cell preparations from whole blood, urine, liquor, pleural fluid, pericardial fluid, peritoneal fluid, synovial fluid, and bronchoalveolar lavage.

16. The method according to any one of claims 5 to 13, wherein the solution contains a mixture of non-methylated human genomic DNA and methylated human genomic DNA.

17. The method according to any one of claims 12 to 18, wherein separation is achieved with the aid of a filter which filters out corresponding DNA-protein complexes.

18. The method according to claim 17, wherein the protein is immobilized on a filter matrix.

19. The method according to any one of claims 5 to 18, wherein as a step d) after step c), the DNA containing non-methylated CPG motifs is furthermore amplified.

20. The method according to claim 19, comprising the steps of:

    a) isolating the DNA containing non-methylated CPG motifs from the protein-DNA complex,
    b) denaturing the double-stranded DNA,
    c) hybridizing the individual strands of the DNA with complementary primers,
    d) generating double-strand fragments via reaction with polymerases, and
    e) repeating these steps up to the desired degree of amplification.

21. The method according to claim 19, comprising the steps of:

    a) cloning the isolated DNA sequences containing non-methylated CPG motifs into vectors,
    b) transforming suitable host cells with these vectors,
    c) cultivating these transformed cells,
    d) isolating the vectors from these cells, and
    e) isolating the DNA.

22. A kit for separating and/or enriching DNA containing non-methylated CPG motifs by means of a method according to any one of claims 5 to 21.

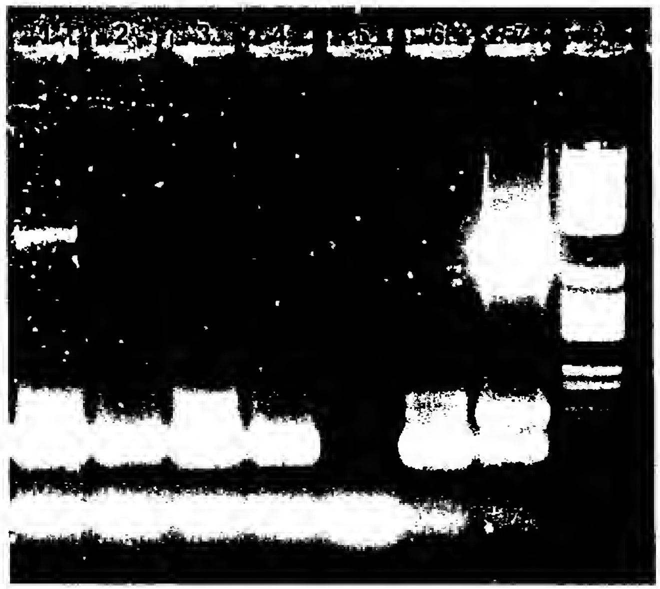
23. A test kit for detection of DNA containing non-methylated CPG-motifs by means of a method according to any one of claims 5 to 21, using one or several sets of specific primers.

24. The kit according to any one of claims 22 and 23, wherein the protein according to SEQ ID No. 1 is contained.

25. The kit according to claim 24, wherein the protein according to SEQ ID No. 1, coupled to a matrix, is contained.

Figure 1

Results of PCR after enriching prokaryotic DNA from DNA mixture of *Staphylococcus aureus* and human DNA by using coupled CpGbP-181 protein on CNBr sepharose

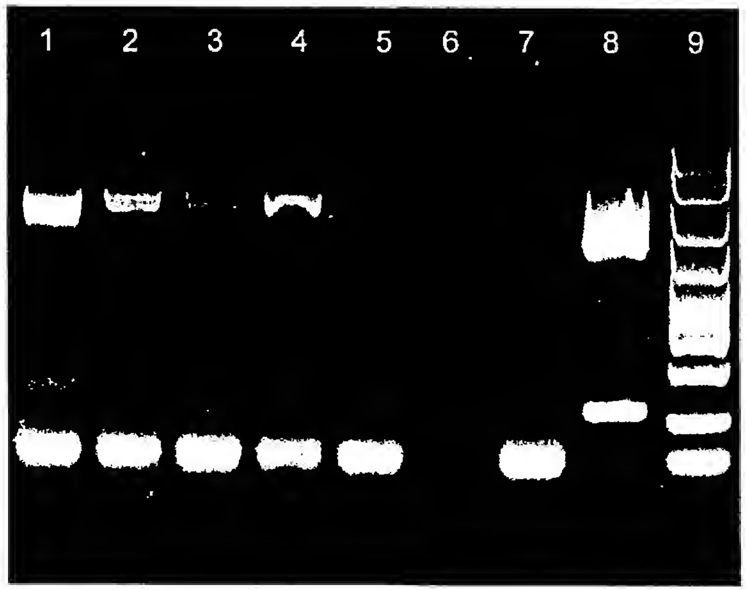


Legend:

1 E_1 (E = elution fraction)
2 E_2
3 E_3
4 E_4
5 E_5
6 before column
7 pos. control
8 pGEM marker

Figure 2

Results of PCR after enriching prokaryotic DNA from DNA mixture of *Staphylococcus aureus* and human DNA by using coupled CpG-181 protein on AH sepharose



Legend:

1 E_1 (E = elution fraction)
2 E_2
3 E_3
4 E_4
5 E_5

6 negative control
7 before column
8 positive control
9 BIORAD marker

SIRS-Lab GmbH
Attorney's file: PAT 3696/031

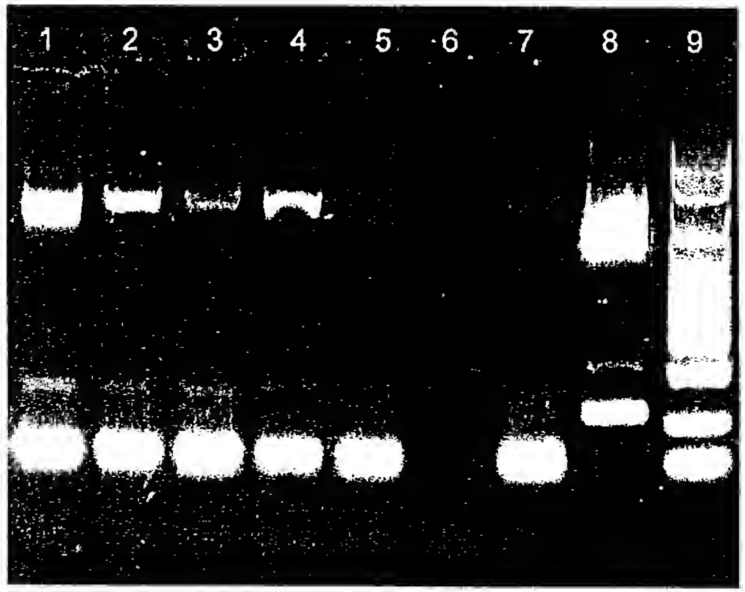
January 14, 2005
H/18/kt

Abstract

The invention relates to a method of enhancing the binding capacity and efficiency of a protein which specifically binds DNA containing non-methylated cytidine-phosphate-guanosine dinucleotides (CpG motifs) of a DNA, to the use of the method of separating and/or enriching DNA containing non-methylated CpG motifs, as well as to a kit for carrying out the method.

Fig. 2

Results of PCR after enriching prokaryotic DNA from DNA mixture of *Staphylococcus aureus* and human DNA by using coupled CpG-181 protein on AH sepharose



Legend:

1	E_1 (E = elution fraction)	6	negative control
2	E_2	7	before column
3	E_3	8	positive control
4	E_4	9	BIORAD marker
5	E_5		